ON THE SIMILARITY BETWEEN BLOOD-PLATELETS AND CERTAIN HEMATOZOA.*†

(WITH PLATE I.)

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IT is a well-known fact that students of hematozoa have from time to time announced the discovery of new blood-parasites, and later have found them to be only normal constituents of the blood. Being aware of this fact and prompted by recent investigation upon *Bàbesia* (*Piroplasma*), I am led to make a statement of certain facts which have come under my observation in the course of investigations on a flagellate occurring in the stomach of the sheep tick, *Melophagus ovinus*.

The mode of transmission of *Babesia* has been a matter of discussion ever since the discovery of the parasite. Schaudinn's (1904) hypothesis that the development takes place in a similar manner to *Halteridium* has been well supported, not only by his own observations, but by experiments of other workers. He states that he found on blood films prepared late in the afternoon by Weber and Kossel from a cow kept in the dark and dying of piroplasmosis, flagellates in which both a blepharoplast and a nucleus could be made out. He then restained and studied old streak preparations made in 1899 from the intestinal contents of ticks which had fastened themselves to hemoglobinuric oxen. Trypanosoma-like stages similar to the above were found.

Working on this hypothesis, Rogers (1904) found that flagellates developed in cultures made by putting blood of patients suffering from certain cachexial fevers and kala-azar into sodium-citrate solutions. His best flagellate stages were obtained in one culture after an incubation of 24 hours at a low temperature. Judging from his figures alone, one could hardly doubt that they represent real trypanosome stages. Still stronger evidence for the existence of a flagellate stage in some insect host is afforded by the statement in a later paper (1905), that an acid solution similar to the conditions existing in the stomach of certain insects is the most conducive to the development of flagellates.

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In a very recent paper (1907) he seems to have shown conclusively that the parasite causing kala-azar does have a flagellate stage. From sodium-citrate cultures he obtained rosettes of dividing flagellates with long flagella centrally located. When the cultures were first made the parasites were small and it was hard to distinguish them from blood-platelets. But later, growth and flagellation took place and by successive divisions the rosettes originated.

Kinoshita (1907) in a paper on *Babesia canis* draws comparisons between this parasite and *Plasmodium*. He claims, although there is room for some doubt, to have found schizogony and gametogony, and lays considerable emphasis upon the presence of nuclear dimorphism. Furthermore, in sodium-citrate cultures he found flagellates, the flagella of which took origin in the blepharoplast and measured in one case 15 μ in length.

The works of Koch (1906), Kleine (1906), and Christophers (1907), do not support the hypothesis of a flagellate stage. Further mention of Koch's and Kleine's papers will be made later. Christophers followed the life-cycle of *Piroplasma canis* through the tick, but neither mentions nor figures any flagellate stage. It is even more striking that he says nothing about nuclear dimorphism.

According to the observations of Fantham (1907) upon *Piroplasma bigeminum* no flagellate stage occurs, although nuclear dimorphism is clearly present. He says, however, "It is most probable, indeed certain, that a flagellate stage does occur in the life-cycle of the Leishman-Donovan body, and may be expected in the alimentary tract of a blood-sucking Arthropod, namely, the bedbug, as suggested by Rogers and now being worked out by Patton. On the other hand, a flagellate stage appears to be absent in the case of *Piroplasma*."

It seems rather strange that the results of these observers should be so contradictory. Furthermore, it would appear quite improbable that, as Fantham suggests, the Leishman-Donovan body would pass through such an important phase as a flagellate stage, which is not to be found in *Piroplasma*, provided that the two forms are very closely related, as is generally believed.

A statement of my own experience may possibly throw light upon the subject. In my investigation of a herpetomonadine flagellate existing in the alimentary tract of the sheep tick, the mode of trans-

mission from tick to tick at once became a question. The fact that insects are known to be intermediate hosts for various other flagellates suggested the possibility that the sheep tick might act as an intermediate host in this case, and, therefore, that the parasite ought to be found in the blood of the sheep. Accordingly, examinations of the sheep's blood were made, but always with negative results. Knowing Schaudinn's hypothesis and the results of Rogers' experiments, the thought occurred to me that I might be dealing in the tick with the flagellate stage of a piroplasma-like form, possibly *P. ovis*, which I had overlooked in the sheep. Working on this hypothesis, I proceeded to make sodium-citrate cultures of sheep's blood, and was gratified to find that flagellates developed which were quite similar to young stages found in the tick. The same results were obtained from different sheep, both young and old. For a check, cultures of dog's, rabbit's, and human blood were made, giving almost identical results. This led me to a more thorough investigation of the origin of these flagellates, resulting in the discovery that they were nothing more than blood-platelets (thrombocytes). A detailed description of these elements will serve to show the possibility of mistaking them for a flagellate stage of *Babesia* or some other species.

The sodium-citrate solution was made by adding to 1,000 c.c. of distilled water, 5 grm. sodium citrate and 5 grm. common salt. Later it was found, as Rogers also noticed in his cultures, that an acid reaction gives the best results. And so, after sterilization, the solution was rendered slightly acid by the addition of a few drops of hydrochloric acid.

The part of the animal from which the blood was to be drawn was thoroughly washed, in some cases with sublimate, in others with strong alcohol, and then clipped with sterile scissors or punctured with a needle. One or two drops of the blood were allowed to drop directly into a test-tube containing about 2 c.c. of a sodium-citrate solution.

The cultures were examined as quickly as possible after the blood was drawn, generally within five or ten minutes. Normal blood-platelets measuring about one-third the diameter of red blood corpuscles appear from surface view circular, oval, or somewhat ameboid, while from a view at right angles to this they appear spindle-shaped, showing that they are biconvex. It should be noticed that these

normal platelets resemble normal piroplasma forms in size and general shape. A nucleus, and refractive spots, probably vacuoles, can be seen (Figs. 1, 2, and 3). For a more detailed description of the normal platelet and its behavior in blood-clot, reference can be made to the works of Dekhuyzen (1901) and Kopsch (1901). In ordinary blood smears, generally all that can be seen of the platelets is a little granular nuclear material. But after they have been in sodium-citrate solution they can readily be fixed on a slide and stained. My method was to place a drop of the culture upon a slide and after it had evaporated down, but was not entirely dry, to drop on some killing fluid, Zenker's being used with the best results. The specimens could then be stained according to Wright, Giemsa, or with iron hematoxylin.

Cultures examined as quickly as possible after drawing the blood already showed ameboid and flagellate forms. A fact of great importance to the student of hematozoa is that the most of these forms are capable of movement. They were seen to roll over, swing round, and often move for a distance equal to the diameter of two or three red corpuscles. Among the ameboid types there were always present in great numbers platelets with few or several long, sharp, or sometimes blunt, pseudopodia (Figs. 17–24). From the description and figures of Koch and Kleine one must conclude that these forms are very similar to, if not identical with, what they describe as developmental stages of *Piroplasma*. To be sure, Koch found his stages in the stomach of the tick, but this does not disprove the statement, for just such forms were also found in the stomach of the sheep tick after sucking the blood of a sheep. Another form which is often found and which has been figured by Dekhuyzen is spindle-shaped with a long, stiff process at one end and a shorter one at the other (Fig. 4). Many times these forms were somewhat bent, appearing like a quarter-moon. In older cultures most of the platelets that had no pseudopodia were at the rim, either inside or just outside, of a transparent circle about the size of or a little larger than the platelet. Those outside looked as if they had crawled out of a thin envelope. As to their significance, I can only say that they apparently were not degeneration forms, inasmuch as they were still active in their ameboid movements.

The more typically flagellate forms, those with a single flagellum, are perhaps of still more importance, because they so perfectly simulate real flagellates. Although they are found immediately after the introduction of the blood into sodium-citrate solution, the "flagellum" is generally quite rigid except at the very end, where it can be seen to vibrate. Notwithstanding this rigidity, they are seen to move about, roll over, and swing around, these movements probably being the result of the vibrations at the tip of the flagellum. The most motile forms were found in a culture of human blood kept for the first six hours in an ice chest and after that at room temperature for 50 hours. Round or pear-shaped individuals with a flagellum measuring in some cases as much as 20 μ were found in abundance (Figs. 5–12). In the pear-shaped forms the flagellum is at the pointed end. It was very slender, in most cases appearing and moving very much like the flagellum of *Euglena*, often with lashings violent enough to move the red blood corpuscles on coming in contact with them. Instead of being smooth, in some instances the flagella had thickened, knotted portions, which bear a close resemblance to Kinoshita's description and drawings of the flagellates which he found (Figs. 5 and 18). It is important to note that he found the best-developed flagellate with a flagellum 15 μ long in sodium-citrate culture.

The various forms are often found grouped together in couplets, triplets, or in masses composed of as many as a hundred individuals. In this condition they retain their individual motion, rolling over and turning about. I have seen these masses stained with iron hematoxylin so that they had the exact appearance of Kleine's photograph (Kleine, Pl. IV, Fig. 14).

Such masses as these have often been found in the stomach contents of sheep ticks which have fed on sheep's blood. The individuals sometimes were seen to separate and move about, their flagella then becoming plainly visible. Single forms with long waving flagella exactly similar in size, shape, and motion to the platelets in sodium citrate were found. Then, again, the spindle-shaped, moon-shaped, and ameboid forms with several long, stiff processes were observed. Just such a form as represented by Koch (Pl. I, Fig. 14) was found not only in the tick but also in sodium-citrate culture. To eliminate the possibility of confusing the platelets with the herpetomonadine

flagellates, which are generally present in adult ticks, they were studied in young ticks before the latter had become infected.

Flagellation of blood-platelets is not limited to sodium-citrate culture, but may take place in other media. As already mentioned, it occurs in the stomach of the sheep tick; it will take place in sodium citrate without an acid reaction; no less certainly, though perhaps not to the same degree, will it occur in ordinary normal salt solution; and, finally, some of my best results were obtained from a sodium-citrate culture to which was added one-eighth agar-agar. In this last culture a type more definite and constant in structure was found along side the types described above. Unfortunately, however, I overlooked them in the living condition, and did not find them till I examined permanent mounts made from the culture after an incubation of 24 hours. This type is more or less rounded, and has one flagellum originating at a definite point in a kind of notch formed by a little curved projection (Figs. 14–16). In some cases I thought I could recognize features transitional between this type and the other monoflagellate forms. On the average they are larger than the other forms. There is no such variability among them in size and shape as is found in the blood-platelets. The relative position of the chromatin masses in the body is quite constant. With the data at hand, no final opinion is possible, yet the evidence points toward a contamination with monads.

In stained preparations one often finds a most striking, yet doubtless merely coincident, resemblance in nuclear conditions to trypanosome forms. While nuclear dimorphism is not by any means to be found in all blood-platelets, yet it is by no means rare. And when it does occur there is not such marked distinction between the nuclear masses as one sees in trypanosomes, but still as much distinction as many of Kinoshita's drawings would indicate for *Babesia*, in which, he claims, nuclear dimorphism comparable to nucleus and blepharoplast of the trypanosomes is present (compare my figures with his Pl. XII). In blood-platelets a condition exists which should be considered a *false* dimorphism as compared with the trypanosomes or *Babesia*. Thus, it would be an easy matter to mistake such blood-platelets for real flagellates having *true* nuclear dimorphism. Forms represented by Figs. 10 and 17 could be explained by regarding them as division

stages in which the nucleus and "blepharoplast" had divided, interpreting the smaller nuclear masses as blepharoplasts and the larger as nuclei.

The flagella and pseudopodia stain like cytoplasm, and not like chromatin as in the trypanosomes. Koch states that in his forms they stain like cytoplasm, while Kinoshita says his stain like chromatin. If Koch and Kinoshita are both correct in their statements, the evidence indicates that Kinoshita observed a true flagellate form, and further that a possible identity exists between Koch's forms and blood-platelets.

One needs only to compare my drawings with Koch's and Kleine's to be convinced of their similarity in size, shape, and structure. Koch gives no measurements and Kleine only gives measurements of some exceptionally large forms, which, pseudopodia expected, measured 14 μ long by 4 μ broad. The latter are larger than any forms that I found. The scale present on my plate will give the reader a more correct idea of the size than could be given by the use of numerals in the context. In comparing plates it should be noted that my drawings are magnified 800 diameters more than Koch's and Kleine's. In Kleine's photographs one will see that some of the pseudopodia are rigid and blunt, while others are wavy and tapering. (Compare my Figs. 4–24 with his Pl. IV, Figs. 5–14, also Pl. V, Figs. 12–26.) There is great variability in the size and shape of the platelets, but not any greater than is evidenced by Koch's and Kleine's figures. It is not a sufficient answer to the similarity I have shown to say that Kleine used defibrinated blood, and hence blood-platelets were not present in his solutions, for no one has demonstrated that the platelets are entirely removed by defibrination.

Since blood-platelets in various culture media and in the stomach of the tick always develop flagella, move about, and manifest such a marked resemblance in form, size, and structure to *Babesia* and the Leishman-Donovan bodies, investigators must furnish criteria to differentiate between the flagellated platelets and the parasites. Until they have established their position by experiments on normal blood, the correctness of their results can be accepted only with some reserve.

It is rather striking that Rogers and Kinoshita did not find in their sodium-citrate cultures the various forms that I have described,

since such forms are so easily observed in these cultures, and are apparently always present. It is also strange that Koch did not find forms with single, long, vibratile flagella. In my cultures and in the sheep tick these forms were numerous. Kleine, however, figures some with single, thick, and apparently rigid flagella.

It is not my intention to deny the development of a flagellate stage of *Babesia* in cultures and in insect hosts, but merely to call attention to the fact that in cultures of normal blood one can find forms similar, if not identical, to those ascribed to *Babesia* by some of the foremost investigators of this parasite. This similarity may aid in explaining the contradictory results of these investigators.

The evidence which I have presented shows that neither are motion and flagellation exclusive characters of parasites nor will they differentiate them from blood-platelets. Each student will have to determine experimentally how to distinguish the two classes of structures.

This study has been only incidental to another investigation and, therefore, by no means exhaustive. I am indebted to Dr. Henry B. Ward, of the University of Nebraska, for his direction of the investigation.

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EXPLANATION OF PLATE 1.

All figures are magnified approximately 2,800 diameters.

FIG. 1.—Normal blood-platelet from culture of human blood.

FIGS. 3 and 3.—Normal blood-platelets from a 3-hour citrate culture of sheep's blood.

FIG. 4.—Bipolar form from a 24-hour citrate culture of sheep's blood.

FIGS. 5–12.—Pear-shaped platelets with a single flagellum at the pointed end. Figs. 5, 6, and 7 from an 18-hour normal-salt-solution culture of sheep's blood. Figs. 8, 10, and 11 from a 24-hour citrate culture of sheep's blood. Figs. 9 and 12 from a 48-hour citrate culture of human blood.

FIG. 13.—Biflagellated form from a 24-hour citrate and agar-agar culture of sheep's blood.

FIGS. 14–16.—Possibly monads which were accidentally introduced. Taken from a 24-hour citrate and agar-agar culture of sheep's blood.

FIGS. 17–24.—Blood-platelets resembling Koch's and Kleine's figures. Fig. 17 from a 27-hour citrate culture of sheep's blood. Figs. 18, 20, and 24 from a 24-hour citrate culture of human blood. Fig. 19 from an 18-hour-normal-salt-solution culture of sheep's blood. Figs. 21–23 from a 24-hour citrate and agar-agar culture of sheep's blood.

PLATE 1.